Vol. 15 April 1939 No. 4

Bulletin of the Agricultural Chemical Society of Japan.

TRANSACTIONS

Über Zuckerbestimmungen im Zuckerrohrsaft.

Von T. Yoshida, T. Tanabe und K. Yamafuji.

Aus dem Institut für Zuckerforschung in Ta'nan.

(Eingegangen am 10. März 1939.)

In der vorliegenden Arbeit haben wir einige Versuche über die Bestimmungen bzw. Veränderungen des Zuckergehaltes des Zuckerrohrsaftes angegeben.

I. Zur Bestimmungsmethode der Zucker im Zuckerrohrsaft.

Bei der Bestimmung des reduzierenden Zuckers im Zuckerrohrsaft ist der Einfluß der darin befindlichen Saccharose auf die Genauigkeit der Zuckerbestimmung zu berücksichtigen. Bekanntlich wird der Rohrzucker durch Behandlung mit Reagenzien, welche bei der Monosebestimmung gewöhnlich verwendet werden, einigermaßen invertiert. In einer früheren Veröffentlichung (1) haben wir zwei Methoden berichtet, die beim Vorhandensein einer großen Menge Rohrzucker die reduzierenden Zucker zuverlässig bestimmen lassen. Diese Methoden wurden später ergänzt und verbessert (2). Wir haben auch eine Mikromethode ausgearbeitet, welche die Bestimmung einer sehr geringen Menge Zucker ermöglicht (3). In der vorliegenden Untersuchung wurde zunächst versucht, die Ofnersche Methode (4), die oft zur Bestimmung des reduzierenden Zuckers in rohem Rohrzucker benutzt wird, zur Ermittelung des Zuckergehaltes des Zuckerrohrsaftes anzuwenden.

1 ccm frisch gewonnenen Zuckerrohrsaftes wurde mit 0,2 ccm 10% iger Bleiacetatlösung versetzt, geschüttelt und mit Wasser auf 25 ccm verdünnt. Diese Lösung wurde filtriert und zu 15 ccm Filtrat 0,5 ccm einer 10% igen Natriumphosphatlösung zugesetzt. Wieder filtrierten wir die Lösung und verwendeten das erhaltene klare Filtrat zur Zuckerbestimmung.

Zur Ermittelung der gesamten Zuckermenge muß die Saccharose im Filtrat mit Säure vollständig invertiert werden. Um die geeignetste Bedingung für die Inversion zu finden, wurden 50 ccm einer 0,2% igen Rohrzuckerlösung mit 10 ccm Salzsäure von verschiedenen Konzentrationen 30 Minuten im siedenden Wasserbad erhitzt, dann mit Natriumhydroxyd neutralisiert und der entstandene Invertzucker nach der Ofnerschen Methode bestimmt (Tabelle I). Da die Ofnersche Methode in einer schwer zugänglichen Zeitschrift veröffentlicht worden ist, wird hier das Verfahren kurz angegeben.

10 ccm der mit Natriumhydroxyd neutralisierten Lösung werden mit 50 ccm Ofnerscher Kupfersulfatlösung versetzt. Man erhitzt die Mischung in 3 Minuten Kochen und kühlt rasch in fliessendem Wasser ab. Dann setzt man 15 ccm 1 *N* Salzsäure langsam hinzu und anschließend noch 10 ccm 0,032 *N* Jodlösung. Nach 2 Minuten langem Stehen bei Zimmertemperatur wird die Mischung mit 0,032 *N* Thiosulfatlösung gegen Stärke titriert. 1 ccm der verbrauchten 0,032 *N* Jodlösung entspricht immer 1 mg Invertzucker.

Für die Zuckerbestimmung nach der Ofnerschen Methode sind folgende Reagenzien erforderlich:

1. 5 g kristallisiertes Kupfersulfat + 50 g kristallisiertes Natriumphosphat + 10 g wasserfreies Natriumcarbonat + 300 g Seignettesalz in 1000 ccm Wasser.
2. 0,032 *N* Jodlösung: 10 g Jodkalium + 2,05 g Jod in 500 ccm Wasser.
3. 0,032 *N* Thiosulfatlösung: 4 g Thiosulfat in 500 ccm Wasser.

Tabelle I.

Konz. der Salzsäure in *N*	Invertzucker in mg	Konz. der Salzsäure in *N*	Invertzucker in mg	Konz. der Salzsäure in *N*	Invertzucker in mg
0,01	2,74	0,2	4,15	4,0	3,85
0,02	3,58	0,5	4,20	6,0	3,45
0,05	4,08	1,0	4,15	12,0	2,65
0,1	4,10	2,0	4,00	—	—

Bei 30 Minuten langer Erhitzung der Rohrzuckerlösung mit 0,2∼1,0 *N* Salzsäure wird der Zucker fast vollständig invertiert; die berechnete Menge Invertzucker beträgt hierbei 4,21 mg.

Wir erhitzten dieselbe Saccharoselösung verschiedene Zeiten mit 1 *N* Salzsäure auf 100° und verglichen die gefundenen Invertzuckermengen (Tabelle II).

Tabelle II.

Erhitzungsdauer in Min.	Invertzucker in mg	Erhitzungsdauer in Min.	Invertzucker in mg
1	1,25	10	4,20
3	3,55	20	4,20
5	4,10	30	4,15

Aus den Versuchen in Tabelle II geht hervor, daß die vollkommene Inversion der Saccharose nach 10 Minuten langem Erhitzen auf 100° stattfindet.

Der reduzierende Zucker im Zuckerrohrsaft kann direkt nach der Ofnerschen Methode bestimmt werden. Zur Ermittelung des Saccharosegehaltes des Saftes muß man die Menge des reduzierenden Zuckers von der auf obenerwähnte Weise bestimmten Gesamtzuckermenge subtrahieren.

Ofner hat in seiner Mitteilung berichtet, daß nach seiner Methode 1 ccm der verbrauchten Jodlösung 1 mg Invertzucker entspricht; dabei wird die verbrauchte

Jodmenge dadurch berechnet, daß man den Titrationswert der 0,032 *N* Thiosulfatlösung von der vorher zugesetzten Menge des 0,032 *N* Jods abzieht. Es ist noch unbekannt, ob dieses Verhältnis auch für die anderen reduzierenden Zucker angewandt werden kann. Bekanntlich fallen die relativen Reduktionswerte der Zucker für die verschiedenen Methoden sehr verschieden aus. Wir haben nun die Glucose in der obenerwähnten Ausführungsweise bestimmt (Tabelle III).

Tabelle III.

Zugesetzte Glucose in mg	0,032 *N* Jod in ccm	Zugesetzte Glucose in mg	0,032 *N* Jod in ccm
2	1,92	6	6,00
4	3,94	8	8,11

Die Tabelle III zeigt, daß das von Ofner für Invertzucker festgestellte Verhältnis: 1 ccm 0,032 *N* Jod =1 mg Zucker sich auch auf die Glucose anwenden lässt.

Die Zusatzversuche in Tabelle IV dienen als Belege für die Anwendbarkeit der Methode auf die Zuckerbestimmung im Zuckerrohrsaft.

Tabelle IV.

	Grade Balling	Rohrzucker in %		Reduzierender Zucker in %	
		Polarisationsmethode	Die von uns benutzte Methode	Luffsche Methode	Die von uns benutzte Methode
Saft Nr. 1	17,1	14,94	14,79	1,070	1,039
Saft Nr. 2	16,6	13,98	13,77	1,570	1,581
Saft Nr. 3	16,9	14,46	14.23	1,660	1,665

II. Über die Veränderungen des Zuckergehaltes des Zuckerrohrsaftes beim Stehen.

Wenn der Zuckerrohrsaft bei Zimmertemperatur stehengelassen wird, so findet allmählich eine Zersetzung der Zucker im Saft durch Enzyme oder Mikroben statt. Für die Reihenanalyse des Saftes ist es notwendig, diese Zersetzung beim Stehen vollkommen zu verhindern. Da im allgemeinen in der Fabrik eine große Anzahl von Säften gleichzeitig analysiert werden müssen, haben wir in dieser Versuchsreihe über die fäulniswidrigen Mittel für den Zuckerrohrsaft eingehend untersucht.

Die frisch geernteten Zuckerrohre wurden 24 Stunden bei Zimmertemperatur stehengelassen und dann mittels einer Presse ausgepresst. Der erhaltene Saft wurde in einen Erlenmeyer-Kolben gebracht, mit einer bestimmten Menge eines antiseptischen Mittels gemischt und alsdann der Kolben gestöpselt. Nach bestimmten Zeiten wurde der Zuckergehalt des Saftes ermittelt. Dabei wurde der Rohrzucker nach der Polarisationsmethode und der reduzierende Zucker nach der Schoorlschen Methode (5) bestimmt.

1. Mercurichlorid.

Tabelle V.

Saft aus dem unreifen Zuckerrohr. Aufbewahrungstemperatur: 28°∼31°. Brix des Saftes: 18.6°.

Aufbewahrungsdauer in Stdn.	0	6	24	0	6	24
$HgCl_2$ in g pro 1 *l* Saft	Rohrzucker in %			Reduzierender Zucker in %		
0	16,36	19,30	15,66	1,05	1,16	—
0,1	16,36	16,36	16,35	1,05	1,08	1,12

Tabelle. VI.

Saft aus dem reifen Rohr. Aufbewahrungstemperatur: 27°∼29°. Brix des Saftes: 22,5°.

Aufbewahrungsdauer in Stdn.	0	6	24	0	6	24
$HgCl_2$ in g pro 1 *l* Saft	Rohrzucker in %			Reduzierender Zucker in %		
0	21,09	21,04	20,85	0,29	0,41	0,90
0,250	21,09	21,09	21,07	0,29	0,32	0,32
0,100	21,09	21,09	21,07	0,29	0,32	0,33
0,050	21,09	21,09	21,07	0,29	0,32	0,33
0,025	21,09	21,08	21,00	0,29	0,32	0,33

Tabelle VII.

Saft aus dem überreifen Rohr. Aufbewahrungstemperatur: 28°∼31°. Brix des Saftes: 14,2°.

Aufbewahrungsdauer in Stdn.	0	6	24	0	6	24
$HgCl_2$ in g pro 1 *l* Saft	Rohrzucker in %			Reduzierender Zucker in %		
0	10,40	10,20	9,20	1,88	1,97	0,18
0,1	10,40	10,40	10,20	1,88	1,89	1,97

Wie aus den Tabellen V, VI und VII ersichtlich ist, wird die Zersetzung der Zuckerarten im Saft durch Zusetzen einer sehr geringen Menge Mercurichlorid vollständig verhindert. Wird das Mercurichlorid nicht zugesetzt, so wird der Rohrzucker allmähich invertiert und es vermehrt sich der reduzierende Zucker. Nach längerer Aufbewahrungszeit vermindern sich aber auch die Monosen. Diese Veränderungen der Zucker sind im allgemeinen beim Saft aus dem überreifen Zuckerrohr stärker ausgeprägt, als beim Saft aus dem unreifen Rohr.

2. *Formalin.*

Tabelle VIII.

Aufbewahrungsdauer in Stdn.	0	6	24	0	6	24
	Rohrzucker in %					
Formalin in g pro 1 *l* Saft	Reifes Rohr			Überreifes Rohr		
0	18,06	18,04	17,69	18,43	18,31	17,25
1,00	18,06	18,06	17,95	18,43	18,31	18,11
0,50	18,06	18,06	17,95	18,43	18,35	18,11
0,25	18,06	18,06	17,95	18,43	18,35	18,11
0,10	18,06	18,05	17,93	18,43	—	—

3. *Mercurijodid.*

Tabelle IX.

Aufbewahrungsdauer in Stdn.	0	6	24	0	6	24
HgI_2 in g pro 1 *l* Saft	Rohrzucker in %			Reduzierender Zucker in %		
0	18,06	18,04	17,69	0,41	0,47	0,48
0,25	18,06	18,06	17,99	0,41	0,40	0,50
0,10	18,06	18,06	17,98	0,41	0,42	0,50
0,05	18,06	18,06	17,98	0,41	0,42	0,49

4. *Natriumbenzoat.*

Tabelle X.

Aufbewahrungsdauer in Stdn.	0	6	24	0	6	24
Na-Benzoat in g pro 1 *l* Saft	Rohrzucker in %			Reduzierender Zucker in %		
0	20,10	20,06	19,86	0,25	0,28	0,34
5,0	20,10	20,02	19,99	0,25	0,27	0,32
3,0	20,10	20,04	20,03	0,25	0,27	0,31
2,0	20,10	20,06	20,04	0,25	0,26	0,31
1,0	20,10	20,10	20,04	0,25	0,28	0,32
0,5	20,10	20,08	20,02	0,25	0,28	0,31
0,1	20,10	20,08	19,92	0,25	0,29	0,24

5. *Natriumsalicylat.*

Tabelle XI.

Aufbewahrungsdauer in Stdn.	0	6	24	0	6	24
Na-Salicylat in g pro 1 *l* Saft	Rohrzucker in %			Reduzierender Zucker in %		
0	18,66	18,64	17,77	0,31	0,34	0,62
3,0	18,66	18,58	18,64	0,31	0,35	0,40
2,0	18,66	18,60	18,55	0,31	0,36	0,41
1,0	18,66	18,64	18,53	0,31	0,35	0,40
0,5	18,66	18,65	18,53	0,31	0,35	0,36
0,1	18,66	18,65	18,65	0,31	0,34	0,36

6. *β-Naphthol.*

Tabelle XII.

Aufbewahrungsdauer in Stdn.	0	6	24	Aufbewahrungsdauer in Stdn.	0	6	24
β-Naphthol in g pro 1 *l* Saft	Rohrzucker in %			β-Naphthol in g pro 1 *l* Saft	Rohrzucker in %		
0	16,85	16,85	15,97	1,0	16,85	16,85	16,81
3,0	16,85	16,85	16,80	0,5	16,85	16,85	16,81
2,0	16,85	16,85	16,81	0,1	16,85	16,85	16,21

7. *Borax.*

Tabelle XIII.

Aufbewahrungsdauer in Stdn.	0	6	24	0	6	24
Borax in g pro 1 *l* Saft	Rohrzucker in %			Reduzierender Zucker in %		
0	19,25	19,21	18,72	0,25	0,27	0,76
2,0	19,25	19,23	19,19	0,25	0,25	0,26
1,0	19,25	19,23	19,11	0,25	0,25	0,31
0,5	19,25	19,23	—	0,25	0,25	0,52

8. *Chloroform.*

Tabelle XIV.

Aufbewahrungsdauer in Stdn.	0	6	24	0	6	24
$CHCl_3$ in g pro 1 *l* Saft	Rohrzucker in %			Reduzierender Zucker in %		
0	19,25	19,21	18,72	0,25	0,27	0,76
1,0	19,25	19,25	19,19	0,25	0,26	0,32
0,5	19,25	19,23	19,02	0,25	0,26	0,32
0,2	19,25	19,23	—	0,25	0,27	—

Wir haben neben den Versuchen in den Tabellen V bis XIV noch zahlreiche diesbezügliche Versuche ausgeführt. Aus diesen Versuchen geht hervor, daß von den untersuchten Mitteln das Mercurichlorid die stärkste antiseptische Wirkung besitzt. Formalin, Natriumbenzoat und Natriumsalicylat haben ungefähr gleiche fäulniswidrige Wirksamkeit. Mercurijodid ist ein starkes Gift; es hat aber eine geringere antiseptische Wirkung als Mercurichlorid. Die Wirksamkeit von Borax und Chloroform als antiseptische Mittel ist geringer als diejenige der obenerwähnten Substanzen. β-Naphthol besitzt eine reduzierende Wirkung; deshalb ist der mit ihm versetzte Zuckerrohrsaft als Material für die Zuckerbestimmung ungeeignet.

Neutrales und basisches Bleiacetat haben auch eine ziemlich starke antiseptische Wirkung aufzuweisen. Bei der Bestimmung des Zuckergehaltes des Zuckerrohrsaftes wird im allgemeinen der Saft mit diesen Reagenzien gereinigt. Wir haben in diesem Versuch zu 100 ccm Zuckerrohrsaft 2 g basischen Bleiacetats zugesetzt und nach bestimmten Zeiten mit 20 cm Rohr den Polarisationsgrad des Saftes ermittelt (Tabelle XV).

Tabelle XV.

Aufbewahrungsdauer in Stdn.	0	6	12	24	48
Polarisationsgrad	69,65	69,65	69,70	69,75	69,80

Wir haben dann zu 50 ccm Zuckerrohrsaft 10 ccm einer 10% igen Bleiacetatlösung hinzugefügt und nach verschiedenen Zeiten mit Natriumcarbonat entbleit. Die Veränderungen der Menge von reduzierendem Zucker im Saft sind in Tabelle XVI wiedergegeben.

Tabelle XVI.

Aufbewahrungsdauer in Stdn.	Saft aus dem reifen Rohr	Saft aus dem überreifen Rohr
	Reduzierender Zucker in %	
0	0,34	0,66
24	0,36	0,70
48	0,38	0,72

Es wurde dann untersucht, ob sich durch Zusatz verschiedener antiseptischer Mittel die Wasserstoffionenkonzentration des Zuckerrohrsaftes verändert (Tabelle XVII).

Die Veränderungen der pH-Werte durch Zusatz der antiseptischen Mittel sind beim Zuckerrohrsaft bedeutend geringer als beim Wasser. Natriumbenzoat oder Natriumsalicylat aber üben auf den pH-Wert des Saftes keine Wirkung aus. Wenn dem Zuckerrohrsaft die anderen Mittel zugesetzt werden, so wird er für die genaue pH-Bestimmung unbrauchbar.

Auf Grund der obenerwähnten Versuchsreihen haben wir die für die voll

Tabelle XVII.

Zugesetztes Mittel in g pro 1 *l* Flüssigkeit	Gefundener pH-Wert		Zugesetztes Mittel in g pro 1 *l* Flüssigkeit	Gefundener pH-Wert	
	Zuckerrohrsaft (pH=5,6)	Wasser (pH=5,8)		Zuckerrohrsaft (pH=5,6)	Wasser (pH=5,8)
0,5 ccm Formalin	4,8	5,0	1 g Na-Salicylat	5,5	6,0
1 ccm Chloroform	5,3	5,0	2 g Borax	6,6	8,8
1 g Na-Benzoat	5,6	6,2	1 g β-Naphthol	5,3	5,5

ständige Konservierung des Zuckerrohrsaftes notwendigen Mengen der fäulniswidrigen Mittel festgestellt (Tabelle XVIII).

Tabelle XVIII.

Mercurichlorid	0,01%	Na-Salicylat	0,05%	Chloroform	0,1%
Formalin	0,05%	Na-Benzoat	0,05%	Borax	0,2%
Mercurijodid	0,05%	β-Naphthol	0,05%		

III. Veränderungen des Zuckergehaltes des Zuckerrohrsaftes durch Bestrahlung mit Sonnenlicht.

Das Zuckerrohr gedeiht bekanntlich nur in der tropischen oder subtropischen Zone sehr gut. Eine Untersuchung scheint daher von großem Interesse zu sein, wie sich die Zuckerarten im Zuckerrohr durch unmittelbare starke Sonnenstrahlen verändern.

In dieser Versuchsreihe haben wir zunächst die Differenz zwischen dem Zuckergehalt des Saftes aus der von Osten und der von Westen bestrahlten Seite ein und desselben im Feld stehenden Zuckerrohrstengels bestimmt. Die Zuckerbestimmung wurde nach der Methode von Hagedorn und Jensen (6) ausgeführt (Tabellen XIX und XX).

Tabelle XIX.

15 Monate altes Zuckerrohr.

Gezählt von unten	Saft aus der östlichen Seite			Saft aus der westlichen Seite		
	Grade Brix	Rohrzucker in %	Glucose in %	Grade Brix	Rohrzucker in %	Glucose in %
3. Knoten	20,6	17,9	1,30	19,8	16,6	1,57
6. "	19,7	17,0	1,90	20,1	16,6	1,93
9. "	20,6	16,8	2,27	20,5	17,6	2,12
12. "	20,9	17,7	2,56	21,2	17,2	2,38
15. "	22,3	20,5	1,31	23,0	20,5	1,15

Tabelle XX.

16 Monate altes Rohr.

Gezählt von unten	Saft aus der östlichen Seite			Saft aus der westlichen Seite		
	Grade Brix	Rohrzucker in %	Glucose in %	Grade Brix	Rohrzucker in %	Glucose in %
3. Knoten	19,2	18,3	1,65	20,2	18,0	1,22
6. ″	22,3	20,9	0,41	21,7	19,7	0,62
9. ″	22,2	19,6	0,46	21,9	20,7	0,37
12. ″	21,1	18,3	0,67	21,1	18,9	0,81

Aus den oben angeführten und noch anderen Versuchen können wir schließen, daß zwischen dem Zuckergehalt der Ostseite des Rohrs und dem der Westseite kein Unterschied besteht.

Wir haben dann untersucht, ob zwischen der Zuckermenge im Saft aus der östlichen Seite eines im Feld stehenden Zuckerrohrs und derjenigen aus der nördlichen Seite desselben Rohrs irgendein Unterschied besteht (Tabellen XXI und XXII).

Tabelle XXI.

15 Monate altes Rohr.

Gezählt von unten	Saft aus der östlichen Seite			Saft aus der nördlichen Seite		
	Grade Brix	Rohrzucker in %	Glucose in %	Grade Brix	Rohrzucker in %	Glucose in %
3. Knoten	21,3	18,1	0,69	21,4	18,1	0,81
6. ″	21,9	17,7	0,69	21,1	18,3	0,67
9. ″	21,5	18,5	0,67	22,5	20,0	0,54
12. ″	23,3	19,9	0,55	23,9	20,2	0,51
15. ″	23,7	22,8	0,50	23,4	21,3	0,26

Tabelle XXII.

16 Monate altes Rohr.

Gezählt von unten	Saft aus der östlichen Seite			Saft aus der nördlichen Seite		
	Grade Brix	Rohrzucker in %	Glucose in %	Grade Brix	Rohrzucker in %	Glucose in %
3. Knoten	19,2	18,3	0,65	20,0	19,0	0,78
6. ″	22,3	20,9	0,41	22,1	21,4	0,46
9. ″	22,2	19,5	0,45	22,4	19,8	0,36
12. ″	21,1	20,1	0,67	21,6	20,1	0,82
15. ″	22,0	20,6	0,28	21,1	20,1	0,46

Wie aus Tabelle XXI und XXII ersichtlich, ist hierbei auch keine Differenz zu erkennen.

Es wurde ferner geprüft, welcher Unterschied zwischen dem Zuckergehalt des Saftes aus der nördlichen Seite eines Rohrstengels und demjenigen aus der südlichen Seite desselben Stengels besteht (Tabelle XXIII und XXIV).

Tabelle XXIII.
13 Monate altes Rohr.

Gezählt von unten	Saft aus der nördlichen Seite			Saft aus der südlichen Seite		
	Grade Brix	Rohrzucker in %	Glucose in %	Grade Brix	Rohrzucker in %	Glucose in %
3. Knoten	19,8	19,0	0,36	19,3	17,9	0,47
6. 〃	19,1	15,5	1,13	18,8	14,9	1,25
9. 〃	20,2	19,0	0,41	19,8	18,5	0,55
12. 〃	19,2	17,3	1,45	18,8	16,0	1,57

Tabelle XXIV.
18 Monate altes Rohr.

Gezählt von unten	Saft aus der nördlichen Seite			Saft aus der südlichen Seite		
	Grade Brix	Rohrzucker in %	Glucose in %	Grade Brix	Rohrzucker in %	Glucose in %
3. Knoten	18,6	16,1	0,07	18,6	15,9	0,07
6. 〃	21,4	18,5	0,06	20,4	18,5	0,07
9. 〃	22,0	21,2	0,12	21,4	19,5	0,18
12. 〃	23,4	19,9	0,04	22,0	19,3	0,06
15. 〃	24,2	23,2	0,07	21,7	19,2	0,06

Tabelle XXIII und XXIV zeigen, daß der Brixgrad und Rohrzuckergehalt des Saftes aus dem schattigen Teil eines im Feld stehenden Zuckerrohrstengels etwas höher sind, als diejenigen des Saftes aus dem Sonnenlicht empfangenden Teil, dass aber die Menge des reduzierenden Zuckers beim letztgenannten Saft größer ist, als beim erstgenannten.

Zusammenfassung.

1. Es wurden Versuche über die Bestimmungsmethoden der Zucker im Zuckerrohrsaft mitgeteilt.

2. Es wurde der Einfluß verschiedener Reagenzien auf die Zersetzung der Zuckerarten im Zuckerrohrsaft untersucht.

3. Es wurde auch untersucht, welche Wirkung die Sonnenstrahlen auf den Zuckergehalt des Zuckerrohrs ausüben.

Literatur.

(1) T. Yoshida, Y. Iwata u. K. Yamafuji; Enzymol., **2**, 342 (1938).
(2) K. Yamafuji u. T. Yoshida; Enzymol., im Druck.
(3) K. Yamafuji u. T. Yoshida; Biochem. Zs., im Druck.
(4) R. Ofner; Zs. Zuckerind. cs. Rep., **53**, 249 (1931).
(5) N. Schoorl; Handb. Pflanzenanlyse, Bd. II, Teil **1**, S. 787.
(6) H. C. Hagedorn u. B. N. Jensen; Biochem. Zs., **135**, 45 (1923).

Studies on the Enzyme in Young Mycelia of *Aspergillus Oryzae.*

By Yoshio OTANI.

From the Zymomycological Institute, Imperial College of Agriculture, Tottori, Japan.

(Received Mar, 7, 1939.)

INTRODUCTION.

There are many kinds of enzymes produced by *Aspergillus Oryzae.* There is no need of giving special references to literature on this subject as the fact is well known.(1~2) All of the literature makes references to enzymes in the full growth of the mycelia mass of this fungus. In this study, the author made experiments especially with young mycelia, the word "young mycelia" being used in the sense of fungus which has been cultivated but on which no spores have yet grown. Recently, especially in Japan, in the industrial use, i. e., in brewing Japanese saké and in manufacture of medicines, young mycelia of this fungus are very largely used. So the author undertook to study the enzymes in the young mycelia of this mold, and he will now describe 13 kinds of enzymes which he found in this experiment.

EXPERIMENT.

Materials and Enzyme Solution.

The materials used in this experiment were prepared in the following manner; the fungus was inoculated in a synthetic solution, and waiting until there was a sufficient growth of mycelia, they were taken off and washed with water, dried at a low temperature, and ground into powder. The enzymatic solution was prepared as follows:— one gram of the above mentioned fungus powder put into 100 cc of distilled water and left for four hours at a temperature of 40°C, the mixture being frequently stirred. The solution was then put through a Zeitz-filter, and the clear solution thus produced was used.

Results of the Experiment.

1. *β-h*-Fructosidase: This enzyme acted on one mol of saccharose and hydrolysed it into one mol of glucose and one mol of fructose. The following method was used to measure the enzyme action: 2% saccharose was used as substrate, 10 cc of it was put into a test-tube, and 5 cc of the enzyme solution was added. In order to keep the solution from putrifying 0.5 cc toluen was added. The mixtare was left in a thermostat at 40°C, for 24 hours. After which it was

put in into a boiling water bath to stop the action of the enzyme. All the fluid was then put into a graduated flask, water was added and the whole solution was increased to a volume of 50 cc, 20 cc of this was taken and the amount of reducing sugar was measured according to the BERTRAND method. From this value that of blank test was deducted and with the remaining number, the power of this enzyme was showen by remaining value.

Table 1.

a : 2% saccharose solution 10 cc + 1% enzyme solution
5 cc + toluen 0.5 cc.
b : 2% saccharose solution 10 cc + 1% boiled enzyme
5 cc + toluen 0.5 cc.
Incubation at 40°C for 24 hours.

Experiment number	0.5% $KMnO_4$ solution (cc)		
	a	b	a—b
1	4.00	0.35	3.65
2	4.00	0.40	3.60

As shown in table 1, there is a strong *β-h*-fructosidase in the young mycelia of *Aspergillus Oryzae*.

2. *α*-*d*-Glucosidase: This is an enzyme which hydrolyse *α*-maltose or *α*-glucoside. In general, there is maltase in this enzyme. A 2% maltose solution was used as a substrate to measure this enzyme. When the enzymatic action was finished, the results were obtained by the BERTRAND method.

Table 2.

a : 2% maltose solution 10 cc + 1% enzyme solution
5 cc + toluen 0.5 cc.
b : 2% maltose solution 10 cc + 1% boiled enzyme
5 cc + toluen 0.5 cc.
Incubation at 40°C for 24 hours.

Experiment number	0.5% $KMnO_4$ solution (cc)		
	a	b	a—b
1	10.95	9.30	1.65
2	10.95	9·20	1.75

As seen from the above table, *α*-*d*-glucosidase can be clearly demonstrated in this enzyme solution.

3. *β-d*-Galactosidase: This enzyme is also called lactase. It decomposes lactose into galactose and glucose. We used as a substrate a 2% lactose solution. As mentioned above, the enzymatic decomposition product was measured by the Bertrand method with the following results:

Table 3.

a: 2% lactose solution 10 cc + 1% enzyme solution
5 cc + toluen 0.5 cc.
b: 2% lactose solution 10 cc + 1% boiled enzyme
5 cc + toluen 0.5 cc.
Incubation at 40°C for 24 hours.

Experiment number	0.5% $KMnO_4$ solution (cc)		
	a	b	a—b
1	10.30	9.00	1.30
2	10.30	9.00	1.30

As indicated in table 3, this enzyme solution contained very little *β-d*-glucosidase.

4. *β-d*-Glucosidase: There is emulsin, in general, in this enzyme. In this experiment, amygdalin was used as substrate.

Table 4.

a: 2% amygdalin solution 10 cc + 1% enzyme solution
5 cc + toluen 0.5 cc.
b: 2% amygdalin solution 10 cc + 1% boild enzyme
5 cc + toluen 0.5 cc.
Incubation at 40°C for 24 hours.

Experiment number	0.5% $KMnO_4$ solution (cc)		
	a	b	a—b
1	18.2	0	18.2
2	18.2	0	18.2

As noted in the above table, emulsin in this fungus enzyme worked very powerfully.

5. *α*-Amylase: In order to find out whether this enzyme was present or not, a starch solution was used, and the experiment was carried out through iodine reaction. 5 cc of this enzyme solution was added to 10 cc of 2% soluble starch solution, and was left at a temperature of 40°C for one hour. Then, iodine was added to the solution which gave it a red-brown colour, when a boiled solution of

this enzyme was used, and iodine added, it showed a blue colour. Thus we concluded that this fungus enzyme contained strong α-amylase enzyme.

6. β-Amylase: This enzyme is one which saccharifies starch. So we used 2% soluble starch as substrate and allowed this enzyme to act on it. The amount of sugar produced was measured by the Bertrand method.

Table 5.

a: 2% soluble starch solution 10 cc + 1% enzyme solution 5 cc + toluen 0.5 cc.
b: 2% soluble starch solution 10 cc + 1% boiled enzyme 5 cc + toluen 0.5 cc.
Incubation at 40°C for 24 hours.

Experiment number	0.5% $KMnO_4$ solution (cc)		
	a	b	a—b
1	9.70	5.00	4.70
2	9.60	5.00	4.60

Table 5 shows that this enzyme solution showed strong β-amylase.

7. Papain type protease: This enzyme is one of the proteinase which has about pH 5.2 optimum hydrogen ion concentration. In our experiment, we used gelatin and casein as substrates on which we allowed this enzyme to work. Then we measured the amino acid produced by the Sörensen method, with the following result:—

Table 6.

a: 2% gelatin solution (or 4% casein solution) 10 cc + 1% enzyme solution 5 cc + toluen 0.5 cc......pH 5.2.
b: 2% gelatin solution (or 4% casein solution) 10 cc + 1% boiled enzyme 5 cc + toluen 0.5 cc......pH 5.2.
Incubation at 40°C for hours.

Substrata	Experiment number	$N/10$ $Ba(OH)_2$ solution (cc)		
		a	b	a—b
Gelatin	1	0.70	0.45	0.25
〃	2	0.70	0.50	0.20
Casein	1	1.20	0.60	0.60
〃	2	1.25	0.60	0.65

In the above table, it can be clearly seen that in this enzyme solution, there is papain type proteinase.

8. Peptide decomposing enzyme: This peptide decomposing enzyme is one which decomposes peptone at about pH 8.0. Using peptone as a substrate of this enzyme, we measured the amino acid produced after the reaction, through the Sörensen method.

Table 7.

a : 2% peptone solution 10 cc + 1% enzyme solution 5 cc + toluen 0.5 cc.
b : 2% peptone solution 10 cc + 1% boiled enzyme 5 cc + toluen 0.5 cc.
Incubation at 40°C for 24 hours.

Experiment number	$N/10$ $Ba(OH)_2$ solution (cc)		
	a	b	a—b
1	3.80	1.95	1.90
2	3.85	1.95	1.95

As shown in the above table, it can be easily seen that in this enzyme solution, there is peptide decomposing enzyme.

9. Lab : In order to test the lab enzyme, the following two methods were used.

1st experiment :— Using fat free milk, pasteurized.

Table 8.

a : Fat free milk 10 cc + 1% enzyme solution 5 cc + toluen 0.5 cc.
b : Fat free milk 10 cc + 1% boiled enzyme 5 cc + toluen 0.5 cc.
Incubation at 40°C for 24 hours.

Experiment number	a	b
1	coagulated	not coagulated
2	coagulated	not coagulated

2nd experiment :— Using casein solution.

1.5 g casein was dissolved in 25 cc of sodium bi-phosphate solution, and was mixed with 25 cc of 0.4% calcium chloride solution, the mixture being used as substrate.

Table 9.

a : Casein solution 10 cc + 1% enzyme solution 5 cc + toluen 0.5 cc.
b : Casein solution 10 cc + 1% boiled enzyme 5 cc + toluen 0.5 cc.
Incubation at 40°C for 24 hours.

Experiment number	a	b
1	coagulated	not coagulated
2	coagulated	not coagulated

As shown by the experiment, in this enzyme solution, there was lab coagulating milk and casein.

10. Urease : In 10 cc of 2% urea solution was added 5 cc of this enzyme solution and 0.5 cc of toluen. This was kept, for a whole day, in a bath at a constant temperature of 40°C. When NESSLER reagent was added, it produced a

clear reddish brown colour. By this, we could see that there was urease in this enzyme solution. In addition we used as blank test, a boiled solution of this enzyme, but the reaction was negative.

11. Peroxidase: 5 cc of 1% pyrogallic acid was mixed with 5 cc of 3% hydrogen peroxide and to this was added 5 cc of this enzyme solution. When this was left in a room at a temperature of ca. 20°C, purpurgallin crystals were clearly seen. But when boiled enzyme were used as control test, the reaction was negative.

12. Indophenolase: To this fungus enzyme, a few drops of "Nadi" reagent were added. After 30 minutes, or an hour, a rose-red or red-violet colour appeared, but the blank test was negative.

13. Catalase: 1% hydrogen peroxide was used as substrate and was added to the enzyme solution. Using permanganate solution, the reaction was tested.

Table 10.

a: 1% hydrogen peroxide solution +10 cc enzyme solution 5 cc.
b: 1% hydrogen peroxide solution +10 cc boiled enzyme 5 cc.
Incubation at 40°C for 30 minutes.

Experiment number	0.5% $KMnO_4$ solution (cc)		
	a	b	a—b
1	1.90	0.30	1.60
2	1.90	0.30	1.60

As shown in the above table, this enzyme certainly has catalase action.

SUMMARY.

This study was made in order to test the enzymes which are produced by young mycelia of *Aspergillus Oryzae*. It was found that there were 13 kinds of enzymes, i. e., β-*h*-fructosidase (saccharase), α-*d*-glucosidase (maltase), β-*d*-galactosidase (lactase), β-*d*-glucosidase (emulsin), α-amylase, β-amylase, papain type protease, peptide decomposing enzyme, lab, urease, peroxidase, indophenolase and catalase.

Among these, peroxidese and indophenolase were weak.

A report will probably be made later of enzymes other than those noted above.

BIBLIOGRAPHY.

(1) Thom, C., and Church, M.: The Aspergilli, 59, (1926).
(2) Buchanan, R. E., and Fulmer, E. I.: Physiology and Biochemistry of Bacteria, III, 33 ~157 (1930).

Studies on Vitamin B_2 Complex.

III—Further Studies on the Growth-promoting Factor in Liver Extract.

By Ume Tange.

From the Institute of Physical and Chemical Reserch.

(Received Oct, 20, 1939.)

In the previous paper,⁽¹⁾ dealing with the vitamin B_2 complex, I presented the evidence for the existence of growth-promoting factor (or factors) which could be separated from liver extract by treating its concentrated aqueous solution, after an exhaustive adsorption with acid clay, with large volumes of a mixture of alcohol and ether. This factor may be similar to or identical with factor W described by Frost and Elvehjem.⁽²⁾

In this paper I wish to present further proof that the profound growth-stimulating property of the alcohol-ether insoluble fraction is due to a factor distinct from riboflavin and to give some properties.

Experimental.

The basal ration described previously was slightly modified, and it has the following composition, supplemented daily with 1 drop of cod liver oil:

Purified fish protein	20%
Dextrinized corn starch	53
White corn	15
Butter fat	8
McCollum's salt	4

From the statement of Frost and Elvehjem,⁽²⁾ we can assume that this ration supplies sufficient amounts of vitamins B_4 and B_5, but is low in flavin and factor W. If flavin were only deficiency in this ration, normal growth should result when flavin is added. No such result appeared as seen in Fig. 1 (b). On the other hand, the striking effect on the growth was obtained by supplementing the liver concentrate to flavin (Fig. 1 (c) and (d)). If the liver growth factor was alone added, there was good growth for some interval, but followed thereafter by decline. At this condition, if flavin was added, there was immediate resumption of growth. If both flavin and liver growth factor were added from the beginning to the diet, there was profound growth response. These results demonstrated very clearly that there is a remarkable supplementary action between flavin and liver growth factor in the nutrition of rats (Fig. 2 (b), (c) and (d)).

Methods and Results.

Young rats weighing between 40 and 50 g were placed on the basal diet and each received daily 20 γ of vitamin B_1 hydrochloride. After being constant in weight for several days, the rats received the test doses of the different fractions supplemented by 20 γ of flavin daily. The control animals received flavin only. Three to six rats were used for each test and growth was observed for a period of six weeks. Some variations had been noted occasionally in the growth responses of rats from different litters, but within a litter, growth responses were fairly regular.

Flavin was prepared from dried egg-white powder extracting with a mixture of methanol-water (3 : 1), and concentrating the extracts under diminished pressure. The amounts of flavin in the concentrate thus obtained was estimated as lumiflavin by Pulfrich's photometer. This raw flavin was provided for rats at the level corresponding to 20 γ of pure flavin.

Preparation of the growth factor from liver extracts.

(a) Koehn and Elvehjem,(3) Macrae and Edgar(4) have reported that growth promoting factor for rats can be extracted from liver concentrate with amyl alcohol. The following procedure, therefore, was carried out: 300 cc of liver extract,(1) free from flavin (1 cc corresponding to 3 g of dry liver) previously treated with acid clay at pH 3.0, was adjusted to pH 1.4 with H_2SO_4 and extracted five times with 500 cc of amyl alcohol. The amyl alcohol solution was shaken three times with 400 cc of 10% NaOH. The alkali extracts were combined and, after neutralizing with HCl, the amyl alcohol was completely removed in vacuo. The effect, however, of the amyl alcohol extract on animal growth, as seen in Fig. 2 (a) and (a′), was not so good as that with acidified aqueous acetone extract obtained by the method described in the following: Two litres of the liver concentrate* (1 cc equivalent to 2 g of fresh beef liver), previously extracted with a mixture of methanol-water, acidified with H_2SO_4, and then treated with acid clay to remove flavin, having an acid reaction of a pale green-yellowish colour was treated twice with 100 g of acid clay each time to remove a trace of flavin which might remain in the solution, then the filtrate from acid clay adsorbates was neutralized with NaOH, and concentrated to about 200 cc in vacuo. Into the dark thick solution, 92% ethyl alcohol was added and the resulting precipitates were separated from the clear alcoholic solution. After similar treatment was repeated twice more, the combined alcoholic solution was evaporated. The 92% alcohol-soluble liver fraction was now placed in 300 cc of 0.2% HCl aq. solution and 2700 cc of acetone was added with constant stirring. The acetone fraction was decanted off and the residue was treated in the same way with a mixture of 600 cc of 0.2% HCl aq. solution and 900 cc of acetone. The similar procedure was repeated five times more. The acetone was recovered from the combined extracts by distillation. The resulting dark, aqueous solution was diluted with distilled water, considerable denatured protein separated out. This was filtered and the clear brown solution free from flavin was concentrated to about 200 cc for use and stored in the refrigerator.

---

* The liver concentrate used as the starting material was kindly provided by Dr. F. Inukai, to whom I wish to express my great indebtedness.

Some experiments of the chemical properties of the growth-promoting factor.

1. Adsorption on charcoal — As I found that the acidified aqueous acetone filtrate contained the active principle, charcoal adsorption according to the method of Edgar et al.(5) was carried out: The solution was adjusted to pH 2.5 with HCl and treated with charcoal. The charcoal was washed with 0.1 *N* HCl and then eluted with 0.25 *N* NaOH, being allowed to stand overnight in the ice box. The elution was repeated once more. The eluate and filtrate were fed by separatory or combination at high levels; neither of them showed any appreciable growth-stimulating effect (Fig. 3 (a)).

2. Precipitation with acetone — The 92% alcohol-soluble fraction diluted with water of half volume of the solution was mixed with acetone. After standing overnight in the cold, the acetone solution was decanted from the gummy precipitates. After repeating similar treatment once more, the combined acetone was removed in vacuo. The greater part of activity was found in the soluble part, whilst the precipitates appeared inactive (Fig. 3 (b)).

3. Phosphotungstic acid precipitation — 60 cc of the 92% alcohol-soluble fraction diluted with water, acidified with H_2SO_4 to pH 1, and a 20% phosphotungstic acid solution was added until there was no further precipitate. After standing 24 hours with occasional stirring, the precipitates were filtered off. The filtrate, after removal of the excess phosphotungstic acid by the usual means, was found to have vitamin activity approximately equal to that of the starting alcohol-soluble fraction. The phosphotungstic precipitate was decomposed by H_2SO_4 and its filtrate was also tested, showing negative results (Fig. 3 (c)).

4. Barium precipitation — 100 cc of the acetone filtrate were diluted with an equal volume of distilled water and treated with a solution of 10 g of barium acetate in 25 cc of H_2O and the formed gummy precipitates were removed by filtering. The filtrate made alkaline to phenolphthalein with a cold, saturated barium hydroxide solution, and the slight precipitate was removed as before. The filtrate was then mixed with a 3-fold volume of alcohol and the formed flocculous precipitate centrifuged out. This precipitate was immediately dissolved in 50 cc of H_2O. After removal of barium quantitatively with sulphuric acid, the concentrate was stored in the ice-box. The alcoholic filtrate was

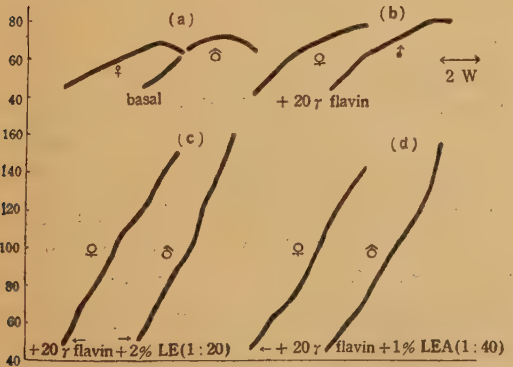

Fig. 1, Growth curves for rats on basal ration (a), basal ration + flavin (b), basal ration + LE (c) and basal ration + LEA (d). LE and LEA are liver concentrate, free from flavin, and 92% alcohol-soluble liver extract, respectively. (1 : 20) and (1 : 40), show that 1 part each of the preparation corresponds to 20 and 40 parts of fresh liver, also freed of barium with sulphuric acid and prepared for assay. The feeding results of these fractions singly and together were given in Fig. 3 (d).

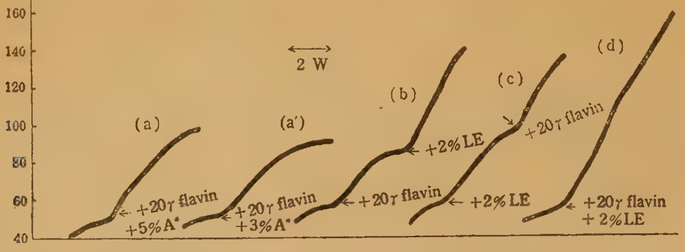

Fig. 2. Growth curves of rats receiving flavin alone, liver concentrate free from flavin, the combination of the two, and flavin + amyl alcohol extract of liver = A* (1 cc of A* is equivalent to 3 g of dry liver).

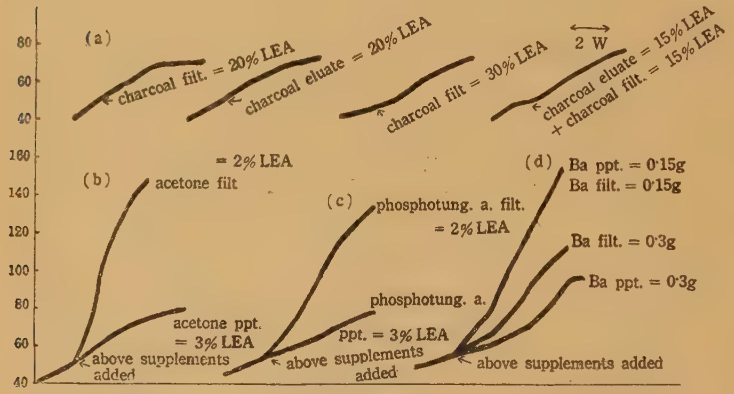

Fig. 3. Growth curves showing the effect on rats receiving various fractions from liver extract.

Can nicotinic acid replace the liver growth factor?

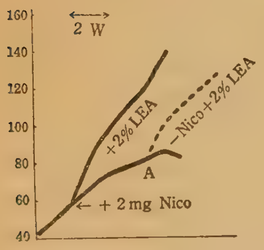

Fig. 4. Growth curves showing the inability of nicotinic acid. At point A, LEA is substituted for nicotinic acid.

Recently the importance of nicotinic acid and its amide in the biological process is recognized, and there is some evidence that the acid or its amide has a growth-promoting action for rats or pigeons on various diets deficient in some factors of the vitamin B complex, while the others claim that these pyridine compounds have no significant effect on the growth of rats receiving vitamin B_1 and flavin as source of vitamin B complex. It is interesting, therefore, to find if nicotinic acid can replace the growth factor in liver extract. The experiment was carried out as follows: The rats received the basal diet supplemented with vitamin B_1. When they ceased to grow, the rats were divided into two groups. One group of the rats received 20 γ of flavin and 2 mg of nicotinic acid daily, while the other group was given, in addition to 20 γ of flavin daily, 2% of the 92% alcohol-soluble liver extract. The results in Fig. 4 showed that nicotinic acid had no significant increase in the growth rate and the acid could not be substituted for the growth factor found in the liver extract.

DISCUSSION.

The observations described above confirm the previous finding(1) that in addition to vitamins B_1, B_4(?), B_6 and flavin, the rat requires still another factor (factor W?) to attain the maximum growth. This factor can be prepared from liver concentrate by dissolving in 92 per cent ethyl alcohol and treating the alcohol-soluble fraction with acidified aqueous acetone. The active factor is not adsorbed by acid earth at pH 1.4 and 3.0 or by charcoal at pH 2.5. The factor is readily soluble in ethyl alcohol and partially precipitated by barium salt, but not by phosphotungstic acid. Recently Edgar and Macrae(5)(6) described that the yeast-extract filtrate factor for rat is probably acidic, since it is precipitated by barium hydroxide in 90 per cent alcohol. In a recent paper Frost and Elvehjem(2) reported evidence suggesting a relation of factor W to pyridine nucleotides.

The comparison of the properties of the fraction, as already stated, in an aqueous acetone solution indicates that the factor promoting growth in rats is probably identical with factor W described by Frost and Elvehjem.(2)

The biological importance of the pyridine compounds, was recently recognized by the demonstration of Warburg and Eular that nicotinamide is the active group in certain coenzymes. Funk and Funk,(7) Frost and Elvehjem(2) showed that nicotinamide, and nicotinic acid and adenine nucleotides have growth-promoting action for pigeons and rats on certain rations. I have found, however, that nicotinic acid has no stimutating effect on the growth of rats, in agreement with the results of Edgar and Macrae,(4) who showed that the pyridine compounds cannot replace their yeast-filtrate factor or yeast-eluate factor, although they have isolated nicotinamide from the yeast-eluate fraction. Moreover, these workers demonstrated the presence of a growth factor for rats distinct from nicotinic acid in an amyl alcohol extract of liver and described that this factor may be identical with the yeast-filtrate factor. Dann and Subbaraw(8) reported that nicotinic acid does not cure rat dermatitis or prevent chick dermatitis and concluded that vitamin B_6 and the filtrate factor are both distinct from nicotinic acid. Recently Elvehjem and his co-workers claimed that a factor was isolated from liver and identified as nicotinic acid amide.(9) More recently Elvehjem and his colleagues(10) reported that nicotinic acid or its amide has no curative power on chick dermatitis and that liver extract contains a factor necessary for the prevention of a typical dermatitis in chick which is separate and distinct from vitamin B_1, vitamin B_6, flavin, nicotinic acid, and factor W.

Regarding the nature of the growth factor (factor W?), I wish to cite the claim of Frost and Elvehjem(2) on the relationship between factor W and pyridine nucleotides and on the chemical behaviour of the factor towards mercury and barium salts which indicated a similarity to the coenzymes. We must consider, however, the comment of Peters(11) who stated: "at present results on factor W are difficult to reconcile with other finding owing to the non-synthetic nature of the diet. Vitamins B_1 and B_6 were provided in the form of white corn composing 12 per cent of the diet which, it is stated by Frost and Elvehjem, also contain small yet adequate amount of the filtrate factor". Further experiments are, therefore, needed with regard to the nature of the growth-stimulating factor in liver.

SUMMARY.

1. A method for separation and concentration from liver extract of the growth-promoting factor (or factors) distinct from flavin is given.

2. The factor (or factors) is readily soluble in ethyl alcohol, and the addition of a large volume of acetone to the aqueous solution cannot precipitate the active factor. The factor is not precipitated by phosphotungstic acid, but partially by barium. It is not adsorbed by acid clay or charcoal at pH 1.4 and 2.5.

3. Normal growth is induced in rats when both flavin and liver growth factor are added to the basal ration.

4. Nicotinic acid cannot replace the active factor, as tested on growth of rats.

5. Comparison of the properties of the factor indicates that it is distinct from riboflavin, but may perhaps be identical with factor W described by Frost and Elvehjem.

I wish to thank Dr. U. Suzuki for his continued interest and advice concerning this work. I am also indebted to Miss T. Kaneko for her generous assistance in the preparation of the materials and the feeding of the animals.

(1) Ume Tange: Rikwagaku-kenkyu-jo Iho, **16**, 1058 (1937)
(2) D. V. Frost and C. A. Elvehjem: J. Biol., Chem., **121**, 255 (1937)
(3) C. J. Koehn and C. A. Elvehjem: J. Biol. Chem., **118**, 693 (1937)
(4) T. F. Macrae and C. E. Edgar: Biochem. J., **31**, 2225 (1931)
(5) C. E. Edgar and T. F. Macrae: Biochem. J., **31**, 893 (1937)
(6) C. E. Edgar and T. F. Macrae: Biochem. J., **31**, 886 (1937)
(7) C. Funk and I. C. Funk: J. Biol. Chem., **119**, XXXV (1937)
(8) W. J. Dann and Y. Subbaraw: J. Nutrition, **16**, 183 (1938)
(9) C. A. Elvehjem, R. J. Madden, F. M. Strong, and D. W. Woolley: J. Biol. Chem., **123**, 137 (1938)
(10) O. Mickelsen, H. A. Waisman, and C. A. Elvehjem: J. Biol. Chem., **142**, 313 (1938)
(11) Annual Review of Biochemistry, **VII**, 317 (1938)

ABSTRACTS
from
TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

Besearches on Mechanical Wood Pulp. Part I.

Chemical Analysis of Pinus densiflora, S. et. Z. and Pinus Thunbergii, Parl.

(pp. 305～308)

By Mamoru WATANABE.

(Agricultural Chemical Institute, Kyoto Imperial University Received March. 9, 1939.)

On the Relation between the Nitrogen Compounds of Seeds of Various Crops and the Diastatic Power of their Malts. Part IV.

Studies on Barley and Naked-barley.

(pp. 309～316)

By Hideo KATAGIRI and Narataro MUGIBAYASI.

(Agr. Chemical Laboratory, Kyoto Imperial University; Received Feb. 22, 1939.)

Experiments were carried out with the seeds of 23 species of barley and of 7 species of naked barley.

During the germination, hordein or glutelin nitrogen of all the seeds decreased and soluble nitrogen always increased, although the rate of decomposition of hordein or glutelin nitrogen and the rate of formation of the individual components of soluble nitrogen varied with the species of the seeds.

The activity of maltase in the germinated seeds was found to be quite different according to the species of the seeds.

It was concluded that the saccharifying power of the germinated seeds could be estimated from the amount of albumin nitrogen of ungerminated seeds including barley and naked barley, as was already suggested with the seeds of kaoliang, mung beans and rice (see Parts I, II and III).

Über den Stickstoff- und Aschengehalt der Kaninchenmuskeln.

(ss. 317～322)

Von M. KANDATSU.

(Aus dem Agrikulturchem. Lab. d. Kaiserl. Univers. Tokio, Japan.
Eingeg. am 22, März, 1939.)

On the Retting of Vegetable Fibre Materials.

(pp. 323～330)

By Toshio NAKAHAMA.

(Yamashina Chemical Institute of Kanebo Silk Factory; Received Mar. 8, 1939.)

Part IX— The Selection of Useful Aerobes for the Bacterial Retting of Flax. It was found that an aerobic process was more effective than the natural retting of flax, and ten strains of aerobic bacteria were isolated from the aerobic retting vat.

One strain of bacilli (No. 5) was found to be most effective for the retting of flax, when pure fermentations of flax were carried out with all the bacteria isolated.

Part X— The Characteristics of the Useful Bacteria for the Aerobic Retting of Flax. The useful retting bacteria was found to reveal very much the same natures as *Achromobacter venosum*: spore was not found in the bacterial cells, milk was not changed, gelatin was not liquefied, nitrate was not reduced, indole was not produced by the bacteria, and the cells were motile, possessing long peritrichous flagella. It was, therefore, concluded that the bacteria belong to a kind of *Achromobacter venosum.*

The Bacteriocidal Quality of Sterilized and Non-sterilized Korean Condiments Against Dysentery Bacilli Group.

(pp. 331～336)

By Y. L. PAK. M. D.

Keijo, Chosen. (Seoul, Korea)
Received Feb. 27, 1939.

The kinds of condiments used by Koreans for their daily food are numerous and are rich in taste of hot pepper, salt, and sourness and are used rather in larger quantity when compared with other countries. In consideration of a view that the daily use of such condiments may, possibly, give some favorable influence against various gastro-intestinal diseases, especially against the causative organism of acute infectious disease of the gut, the author had undertaken a study of various condiments which are in common use in Korea as to their sterility and durability over the dysentery bacilli group.

A series of various condiments of about 20 kinds were examined and they are; *Garlic* (Allium scorodoprasum), *black pepper* (Piper nigrum), *hot pepper* (Capsicum annum), *radish* (Raphanus sativus), *ginger* (Zingiber officinale), *mustard* (Brassica cerna), *rock mushroom* (Gyrophova esculent), *Digenea*, peel of Korean citron (Citrus aurantium), *onion* (Allium fistulosum), *mush-room* (Cortineluss shiitake), *soy* (salted soy bean juice), *miso* (soy bean paste), *hot-peppered miso, vinegar, vinegar flavored with citron, honey, sesame oil* (Oleum sessamin indicum), roasted sesame seed and *Korean molasses*, etc. These were divided into two groups; to one group after sterilizing and to the other without sterilizing were added dysentery bacilli of various strains; namely, Shiga, Komagome A, and B. After 30 minutes to 20 days the result was examined by determining the bacteriocidal action in the first group and in the second group, where no sterilization was made, the process reaching the point of sterility and also the phenomena of antagonism with various non-pathogenic bacteria. The summary of the result is as follows;

1. Generally speaking, the condiments of the non-sterilized group possess stronger bacteriocidal power than those sterilized.

2. Black pepper, mustard, and vinegar are found to be most powerful in bacteriocidal action and honey, sesame oil, and rock mush room which were considered to be less bacteriocidal are also found to be almcst equal in their strengths.

3. The condiments found least bacteriocidal are miso, (soy bean paste) mush room, radish and hot-pepper.

4. The dysentery bacilli of Komagome A strain showed weaker resistence to the condiments, whether sterilized or uneterilized, and Shiga and Komagome B strains are about equal in their resistance.

5. The condiments that had been sterilized showed lowered H ion concentration, and at the same time when the sourness and hot peppery taste had become weakened their bacteriocidal quality had, also, become weakened due to some probable change that might have occurred in their volatile substances.

6. Non-pathogenic bacteria attached to the condiments are stronger in resistance over the pathogenic bacteria and they are considered to be useful because of their antagonism against pathogenic organism within the gut.

7. The bacteriocidal property was due to the chemical constituents and hydrogen ion concentration of the condiments. Concerning the former property a further study is needed to ascertain its exact nature.

8. It was made clear in our experiment that mushroom took less than one-half hour to produce sterilization of dysentery bacilli of Komagome A strain, sesame oil about the same time for A and B strains, and Korean molasses the same for A strain, each manifesting its selective action. However, the soy took

2 hours for dysentery bacilli of Komagome B strain, and the peppered miso the same time for Komagome B strain, this also through their selective bacteriocidal actions.

(Author's abstract)

Eine Experimentelle Fabrikation von Geräucherten Hammelfleisch.

(ss. 337～347)

Von Dr. R. Sasaki, M. Kandatsu u. S. Minato.

(Aus dem Agrikulturchemischen Lab. d. Kaiserl. Univers. Tokio, Japan: Eingeg. am 3, März, 1939.)

Carbohydrates of the Bulbs of Allium. IX.

Structure of Scorodose.

(pp. 348～352)

By Yoshijiro Kihara.

(Agricultural Chemical Laboratory, Tokyo Impereial University: Received Mar. 22, 1939.)

Abbau der Aminosäuren durch Aspergillus Oryzae. III. Mitteilung.

(ss. 353～358)

Von T. Uyemura.

(Aus der Agrikult. Chem. Labor. Kaiserl. Univ. zu Tokio.)

α-Ketosäuren wurden als Abbauprodukte von α-Aminosäuren durch einige zum Aspergillus Oryzae gehörende Stämme unter Vergärungsbedingungen gebildet; α-Ketoisocapronsäure aus *l*-Leucin, α-Ketoglutarsäure aus *l*(+)-Glutaminsäure und *p*-Oxyphenylbrenztraubensäure aus *l*-Tyrosin.

1. Abbau von *l*-Leucin.

Zusammensetzung der Nährlösung: Glykose 1.0 g, KH_2PO_4 0.3 g, $MgSO_4$ 0.01 g, $FeSO_4 \cdot 7H_2O$ Spur, *l*-Leucin 0.8 g, dest. Wasser 100 ccm (unter Zusatz von 2%ig. Kreide.) Die gesamte Menge von *l*-Leucin: 34 g.

17 Kolben (250 oder 300 ccm Nährlösung enthaltend) werden geimpft und bei 31°C 26 Tage lang aufbewahrt.

Gewicht der Pilzdecke (nach 26 tägiger Kulturdauer) 13.2226 g,

N-Gehalt der Pilzdecke 3.89%.

α-Ketoisocapronsäure wurde aus ätherischem Auszug von konzentrierter Vergärlösung isoliert. Ausbeute 1.2205 g.

Phenylhydrazon der *α*-Ketoisocapronsäure. Gelbe Nadeln aus Äther-Ligroin. Fp = 132∼3°C. (Unkorr.)

Analyse;	N (%)	C (%)	H (%)
Gef.	12.77	65.35	7.09
Theo.	12.72	65.42	7.33

Oxim der *α*-Ketoisocapronsäure. Weisse Tafeln. Fp = 149°C. (Unkorr.)

Analyse;	N (%)	C (%)	H (%)
Gef.	9.46	50.04	7.18
Theo.	9.65	49.62	7.65

Aus *l*-Leucin wurde noch *d*-Leucinsäure (Ausbeute 0.7782 g) isoliert.

2. Abbau von *l*(+)-Glutaminsäure.

10 g von *l*(+)-Glutaminsäure werden im Wasser suspendiert, mit NaOH neutralisiert und zur Lösung gebracht, dann mit 1000 ccm der unten beschriebenen zusammengesetzten Nährlösung gemischt. Jeder Kolben (insgesamt 5 Kolben), 300 ccm der gemischten Lösung enthaltend, wird geimpft und bei 31°∼2°C 15 Tage aufbewahrt.

Zusammensetzung der Nährlösung: Glykose 15 g, $Na_2HPO_4 \cdot 12H_2O$ 1.5 g, KH_2PO_4 2.0 g, $MgSO_4$ 0.1 g, $FeSO_4 \cdot 7H_2O$ Spur, dest. Wasser 1000 ccm.

pH des Gemisches (nach Sterilisation) 6.0,
pH der Vergärlösung 7.3,
Gewicht der Pilzdecke (nach 15 tägiger Kulturdauer) 3.5348 g,
N-Gehalt der Pilzdecke 5.16%.

Die Vergärlösung wurde zur Analyse verwandt, und es wurden weisse Kristalle aus dem ätherischen Auszug in Wasser ausgelöst. In salzsaurer Lösung wurde mit 2·4-Dinitrophenylhydrazin HCl-Lösung ein gelber Niederschlag von 2·4-Dinitrophenylhydrazon der -Ketoglutarsäure erhalten.

2·4-Dinitrophenylhydrazon der *α*-Ketoglutarsäure; Gelblich färbende Nadeln. Fp = 221°C.

Analyse;	N (%)	C (%)	H (%)
Gef.	17.40	40.98	3.29
Theo.	17.18	40.49	3.07

3. Abbau von *l*-Tyrosin.

3000 ccm zum Versuch gebrachte Nährlösung wurde in folgenderweise zusammengesetzt und in 10 Kolben eingeteilt. (Die gesamte Menge von *l*-Tyrosin: 5.0 g) Glykose 1.50 g, $Na_2HPO_4 \cdot 12H_2O$ 0.10 g, KH_2PO_4 0.20 g, $MgSO_4$ 0.01 g, $FeSO_4 \cdot 7H_2O$ Spur, *l*-Tyrosin 0.50 g, dest. Wasser 100 ccm.

pH der Nährlösung (nach Sterilisation) 5.75,
pH der Vergärlösung (nach 15 tägiger Kulturdauer) 3.90,
Gewicht der Pilzdecke (wie oben) 4.7295 g,
N-Gehalt der Pilzdecke 3.89%.

p-Oxyphenylbrenztraubensäure wurde in weissen Kristallen aus dem ätherischen Auszug isoliert, und dann in 2·4-Dinitrophenylhydrazon herausgesetzt. Ausbeute = 28.9 mg.

2·4-Dinitrophenylhydrazon der *p*-Oxyphenylbrenztraubensäure; Rötlich bräunliche Nadeln aus Äther-Ligroin, Fp = 198°C. (Unkorr.)

Analyse;	N (%)	C (%)	H (%)
Gef.	15.05	49.05	3.10
Theo.	15.55	49.98	3.32

Aus der Vergärlösung von *l*-Tyrosin bildet sich Acetaldehyd in ziemlich reichlicher Menge.

Studies upon the Moulds, Growing in High Concentration of Sulphuric Acid and Copper Sulfate.

(pp. 359~369)

By Tatsuzo Sato.

(Agricultural Chemical Laboratory, Tokyo Imperial University; Received Feb. 27, 1939.)

It is generally believed that most fungi are sensitive even to low concentrations of sulphuric acid and of copper sulfate.

The author has recently obtained four strains of moulds, which grow well in high density of sulphuric acid and copper sulfate.

Three of them, (*Oospora*, *Dematium*, *Penicillium*) are isolated from sulphuric acid solution, containing copper sulfate, which is employed in certain manufactory, and one of them (*Penicillium*) is isolated from copper sulfate solution of Bertrand's reagents.

The properties of the moulds are as follows.

Oospora viscosa nov. sp.

Growth restricted on any culture media except on koji- and malt-extract agar.

Colonies on koji extract agar, smooth, with wax like gloss, deeply wrinkled up and gloss lost when old, cream buff to avellaneous in color, (Ridgway's Color Standard), 8 mm deep, with mycelial bundle (1~2 mm). Young mycelium 45~85 μ by 1.5~1.8 μ, chlamydospore 6~9.5 μ by 2.4~4.5 μ. Dwarfish conidiophore not branched, 10~15 μ by 1~1.5 μ, sterigmata not observed. Conidia ovoid, sausage- or cylinder-shaped, 2.5~3 μ by 1~1.5 μ, aspect smooth, contents slightly granular. No variation on morphological properties in 7% sulphuric acid.

Opt. tem. for growth 28°C, and the inhibition below 14°C or above 37°C, opt. Ph 5.4. The fungus can grow vegetatively in the following concentrations of acids and copper sulfate: 0.05% $CuSO_4$, 7% H_2SO_4, 2.5% HCl, 2% HNO_3; killed in 40% sulphuric acid in 30 minutes, or in 35% sulphuric acid in 60 minutes. No fermentation on carbohydrates.

Dematium pullulans de Bary var. *acidiphylum* nov. var.

Fertile growth only upon koji extract- and malt extract-agar. Colonies on koji extract agar, smooth, very closely felted, 10 mm deep, with wax like gloss, then aerial hyphae produced and gloss lost with age, black to celandien green, finally hair brown. Young mycelium $45 \times 2 \sim 60 \times 2.5\ \mu$. Chlamydospore ($6 \times 5 \sim 9 \times 8\ \mu$) chamois in color. Sometimes grows yeast-like cell ($3 \times 2.5 \sim 4 \times 3.5\ \mu$). Conidipohore grows from mycelium alone, ($5 \times 1 \sim 6 \times 1.5\ \mu$), conidia globose or elliptical ($1 \times 1.5 \sim 1.5 \times 2\ \mu$). No change in morphological properties in 4% sulphuric acid.

Opt. temp. for growth 30°C, and no growth below 14°C or above 37°C. Opt. Ph 5.4. The fungus is able to grow vegetatively in the following concentrations of acids and copper sulfate: 0.1% $CuSO_4$, 5% H_2SO_4, 1% HCl, and killed in 15% sulphuric acid in 30 minutes, or in 10% sulphuric acid in 60 minutes. No fermentation on carbohydrates.

Penicillium cuprophylum nov. sp.

Colonies on Czapeck's soln. agar in the scarcely zonate group, floccose with marginal area of a deep floccose mass of mycelium, 6 mm deep and $5.4 \sim 5$ cm in diameter after 10 days; central areas greenish glaucous with ripening conidia, then turn to pale olive buff, and finally to light mineral gray, with reverse ochraceus-orange. Drops abundant, colorless, on koji extract agar orange yellow. Odor none. Gelatin not liquefied.

Conidiophores $20 \sim 200\ \mu$ by $2 \sim 3\ \mu$, smooth walled and asymmetrically branched; branches $15 \sim 20\ \mu$ long; metulae $12 \sim 17 \times 2 \sim 3\ \mu$, $2 \sim 3$ in the group: sterigmata about $7 \sim 8\ \mu$ by $1 \sim 1.5\ \mu$, in verticils of about 1 to 5: conidia globose or elliptical $2 \sim 3 \times 1.5 \sim 2.5\ \mu$, smooth; conidial chains tangled. Hyphae $3\ \mu$ in diameter, smooth; in the presence of copper sulfate the older hyphae show a great distortion.

Opt. tem. for growth 28°C, and the inhibition below 0°C or above 37°C, opt. Ph 7.6 or 4.4. Grows vegetatively but does not sporulate in 19% copper sulfate solution. No fermentation on carbohydrates.

Penicillium biforme Thom var. *vitriolum* nov. var.

Colonies on Czapeck's soln. agar 7 mm deep, 7 cm in diameter in 10 days, gelatin not liquefied, floccose, zonate, first white, then central areas dark bluish glaucous with ripening conidia, changing to olive-buff, vinaceous-buff finally to light grayish olive. Reverse warm buff. Drops abundant. Odor none.

Conidiophore with smooth, walls about $20 \sim 140\ \mu$ by $2 \sim 3\ \mu$, asymmetrically branched: branches $15 \sim 25\ \mu$ long, metulae $12 \sim 20 \times 2 \sim 3\ \mu$, $2 \sim 3$ in the group; sterigmata about $10 \sim 12\ \mu$ by $2.5 \sim 3\ \mu$, in verticils of about $1 \sim 5$: conidia globose or elliptical $2 \sim 4 \times 1.5 \sim 3.5\ \mu$, smooth. Conidial chain tangled. Hyphae $3\ \mu$ in diameter, smooth walled; in the presence of 21% copper sulfate the older hyphae show a great distortion.

Opt. tem. for growth 28°C, and the inhibition below 0°C or above 37°C. Opt. Ph 4.4. Grows vegetatively but does not sporulate in 21% copper sulfate solution. No fermentation on carbohydrates.

On a New Polypeptide Isolated from Eisenia Bicyclis.
(Part I.)
The Isolation of "Eisenin" and its Hydrolysis Products.

(pp. 370∼376)

By Tosihiko OOHIRA.

(Agricultural Chemical Laboratory, Tokyo Imperial University Received; Apr. 4, 1939.)

A new polypeptide, named "Eisenin", $C_{13}H_{20}O_6N_4$, which gives intensive biuret reaction, has been isolated from the brown alga, Eisenia bicyclis (Kjellm.) Setchell. It crystallises from dilute alcohol in colourless long needles with silky luster and melts at 225∼226° (decomp.), sintering at about 180°. Eisenin is easily soluble in water and difficultly in absolute alcohol and acetone, but insoluble in ether, ethylacetate and other usual organic solvents. Eisenin is laevorotatory, $[\alpha]_D^{14°} = -54.3$, in aqueous solution. It gives an acid reaction and tastes sour. Eisenin contains one free carboxyl group which is determined by titration with normal alkaline hydroxide solution and also by preparation of *p*-bromphenacylester.

On hydrolysis with 25% sulphuric acid, "Eisenin" yields *d*-glutamic acid (2 mols), *d*-alanine (1 mol) and ammonia (1 mol), as follows:

$$C_{13}H_{20}O_6N_4 + 4H_2O = 2C_5H_9O_4N + C_3H_7O_2N + NH_3$$

The ammonium rest is readily detachable by heating it in weak alkaline solution. The original substance is not ammonium salt, since it gives no Nessler's reaction.

The author suggests, therefore, that the ammonia is formed as an acid amide.

The combination of these components in the polypeptide is now under investigation.

The following method was adopted for the isolation of "Eisenin" from the brown alga.

One kilo of the air dried and powdered alga was extracted three times with boiling 90∼95% alcohol, using 2 liters at a time. A large quantity of mannitol which had separated out on cooling from the united extract was filtered off and the filtrate was evaporated to a small volume (300∼400 cc) under diminished pressure and filtered again from the precipitate formed on evaporation. The filtrate was then evaporated to syrup and extracted with 500 cc of warm water. The solution was kept overnight in a refrigerator and freed of any precipitate that may have formed. The brownish clear solution thus obtained was purified as usual with basic lead acetate by which "Eisenin" was not precipitated. To the purified liquid, after concentration in vacuo to 300 cc, was added saturated aqueous mercuric acetate solution until the yellow precipitate began to occur, keeping the solution always nearly neutral with the addition of 10% aqueous sodium hydroxide solution. An equal volume of 90∼95% ethanol was then added to it and allowed to stand for several hours, whereby the yield of the precipitate substantially increased. The precipitate was collected and decomposed with hydrogen sulphide.

The filtrate from the mercuric sulphide was evaporated under reduced pressure to a small bulk and strong ethanol acidified with sulphuric acid was added to it with constant stirring, whereupon a large amount of crystals separated out. The crystals were collected, washed with ethanol and recrystallized from 80~90% ethanol, yield 8 g.

In an additional experiment with fresh alga, the author was able to isolate the same substance: showing that the polypeptide was not a degradation product formed during the drying of the alga.

The Influence of Monochromatic Lights on the Action of Enzymes.

(pp. 377~384)

By Reitaro MURAKAMI.

(Agricultural College, Utunomiya; Received Jan. 31. 1939.)

[Report XIV.] Especially on the Influence of Ultra-violet Rays on the Action of Enzymes.

(Part 3.) Yeast Proteinase.

The influence of ultra-violet rays on the action of the proteinase in the yeast was investigated by using "Vim Ray" blue lamp. Enzyme solution was prepared as follows: After the liquefaction was completed by the addition of 25 cc of ethyl acetate into 250 g of "Oriental" pressed yeast, 500 cc of water was added. An acid produced during 2 hours was neutralized continuously by the addition of 10% ammonia water and then an ineffective solution for the tryptic and ereptic actions was separated and washed by 1 liter of water. Then the yeast was suspended in 500 cc of water containing toluene and allowed to autolyze at room temperature for 24 hours. An acetic acid solution was added to the autolyzate obtained by the filtration and after the pH of the liquid was converted to 5.0, 5 cc of $N/15$ acetate buffer of pH = 5.0 and 20 cc of alumina cream were added. The proteinase was obtained as a clear solution by the elution of the adsorbate with 44 cc of 5% secondary ammoninm phosphate.

The author added 1 cc of the enzyme solution into the test tubes containing 5 cc of 5% gelatin and 5 cc of citrate buffer, the resulting pH of the solution being 5.1.

The test tubes were placed within tin boxes, with filters at the front windows, and were incubated at 40°C and lighted by "Vim Ray" blue lamp as in the author's previous paper. [Report XII.]

The test tubes were taken out from each box at the intervals of 24, 48 and 72 hours after the incubation and then the end product of the hydrolysis was determined by the former method.

In this experiment, the action of the yeast proteinase was found to be promoted by the visible rays and ultra-violet rays, the latter producing a greater promotion than the former.

[Report XV.] Especially on the Influence of Ultra-violet Rays on the Action of Enzymes.

(Part 4.) Yeast Lipase.

The author has employed "Vim Ray" blue lamp to investigate the influence of ultra-violet rays on the action of the lipase in the yeast. Enzyme solution was prepared by the autolysis for 2 days at room temperature and then for overnight in an ice box after adding 10 cc of toluene and 500 cc of water into 200 g of "Oriental" pressed yeast.

The author added 5 cc of the enzyme solution into the test tubes containing 5 cc of castor oil, which was neutralized with $N/20$ NaOH solution and then treated as in the previous paper. [Report XII.]

The tubes were taken out from each box at the intervals of 8, 16 and 24 hours after the incubation and then the end products, fatty acids of the hydrolysis, were titrated using $N/20$ NaOH solution by Knitz's method.

In this experimeut, the action of the yeast lipase was found to be promoted under iufluence of the visible rays and the ultra-violet rays, but the promotion was greater under the latter than the former.

[Report XVI.] Especially on the Influence of Ultra-violet Rays on the Action of Enzymes.

(Part 5.) Yeast Catalase.

"Vim Ray" blue lamp was used in order to investigate the influence of ultra-violet rays on the action of the catalase in the yeast. Enzyme solution was prepared an follows: After the liquefaction was completed by the addition of 50 cc of toluene into 150 g of "Oriental" yeast within one hour at 40°C, 200 cc of water was added and allowed to autolyze during overnight in an ice box. To the autolyzate obtained by the filtration was added 44 cc of $N/10$ HCl solution and then the total volume of the liquid made to 2000 cc with water and finally 150 cc of alumina cream was added. The catalase was obtained as a clear solution by the elution of the adsorbate with 400 cc of $M/30$ phosphate solution, with $pH = 7.6$.

The author added 1 cc of the enzyme solution into the test tubes containing 20 cc of $N/20$ H_2O_2 buffered with phosphate mixture (pH of the solution was 6.7) and it was treated as in the previous paper. [Report XII.]

The tubes were taken out from each box at the intervals of 5, 10 and 15 minutes after the incubation and the undecomposed hydrogen peroxide in the solution was determined by the titration with $N/20$ $KMnO_4$ solution.

In this experiment, the action of the yeast catalase was found to be inhibited by ultra-violet rays and promoted by visible rays. The action of the enzyme was also promoted under the rays containing both ultra-violet and visible, but the promotion for the enzyme was weaker than by the visible rays alone.

Studies on the Utilization and Digestion by Silkworms of the Carbohydrates contained in Mulberry Leaves.

(pp. 385∼393)

By K. KATO.

(The Sericultural Experiment Institute of Gifu Prefecture; Received Mar. 8, 1939.)

Part VII. The change in the respiration of silkworms when bred on mulberry leaves to which cane sugar was added in different proportions.

As the results of the experiment we found that the activity of respiration is increased when bred on mulberry leaves to which cane sugar was added suitably and decreased when excess sugar was added.

Part VIII. The Change in the metabolism when bred on mulberry leaves to which cane sugar was added suitably and the consideration on the fate of digested sugar.

(1) Total digestion, digestion per 10 hours of the protein and carbohydrate are increased.

(2) The protein, glycogen and fat accumulated in the body per 10 hours are increased.

(3) The consumption of protein is decreased, but carbohydrate consumption is increased.

(4) 8.51% of digested sugar is changed to glycogen, 40.15% to fat and 51.68% is decomposed to CO_2 and H_2O.

Part IX. The digestion of the carbohydrates contained in leaves of several kinds of mulberry trees.

The digestion and digesitibility of carbohydrate, cane sugar and reducing in leaves are positively correlated to the sugar content.

Bergenin from Mallotus Japonicus, J. Mueller.

(pp. 394~396)

By K. Homma.

(Agricultural Chemical Society of Japan; Received Mar. 13, 1939.)

The author has isolated from *Mallotus Japonicus*, J. Mueller, a tree native to Japan and belonging to Euphorbiaceae, a crystalline substance, which shows every property of bergenin, described by A. E. Tschitschibabin and his co-workers.

Futher identification was given by the elemental analysis (C 51.22 proct.; H 5.14 proct.;), methoxyl-number (7.90 proct.;), acetyl-number (38.88 proct.;) in the acetylated substance and lastly by the unaltered melting point with the original specimen of bergenin, which was sent to Assistant Prof. S. Hattori in the Phytochemical Laboratory of the Tokyo Imp. Univ. by Prof. Tschitschibabin himself some years ago.

Über die Bestimmung des Glutathions in Geweben.

(ss. 397~402)

Von Akiji Fujita.

(Aus dem Biochemischen Laboratorium des Kitasato-Instituts. Eingegangen, am 6, Apr., 1939.)

Biochemical Studies on Bakanae Fungus of the Rice. Part III.

Studies on Physiological Action of Gibberellin on the Plant.

(pp. 403~413)

By Teijiro Yabuta and Takeshi Hayashi.

(Imperial Agricultural Station; Received Apr. 8, 1939.)

The physiological action of gibberellin, the active principle which makes the rice seedlings abnormally elongate, upon young shoots of several kinds of plants was tested by means of three different methods as below:—

(A) Water culture experiment.

Young shoots, 5 cm in length, were subjected to the flask culture with the solution containing 0.14∼3.5 mg of gibberellin in 100 ccm water culture solution for about 10 days. The plants used were barley, buckwheat, ryokuto (Phaseolus radiatus, var. typicus, Prain), rape seed, hechima (Luffa cylindrica Roem), tomato, cucumber, cucurbit and morning glory.

(B) Injection experiment.

After Zimmerman and his coworker's experiment,(1) the aqueous solution of gibberellin was injected to the shoots by means of a glass tube drawn to a capillary at one end. The plants used were cucumber, cucurbit, morning glory, soy bean and azuki (Phaseolus anglaris, Wight).

(C) Lanolin application experiment.

After Zimmerman and his coworker's experiment, gibberellin was mixed with lanolin and applied to the basal portion of the shoots of cucumber with a glass rod.

In the above 3 experiments, the plants treated with gibberellin showed abnormal elongation similar to rice seedlings.

Lamna paucicostata, Hegelm, a floating weed found in the rice field, was cultured in solutions containing 0.14∼3.5 mg gibberellin in 100 ccm solution with precautions as to constancy of pH and temperature, avoidance of contamination. After 18 days' culture the plant number, frond number and dry weight were estimated. The reproduction rate and dry weight of the plant cultured in solutions containing gibberellin were much greater than those in control solution.

(1) P. W. Zimmerman, F. Wilcoxon: Contr. B. Thompson Inst. Plant Res., 7, 209∼229, (1935).

On the Koji-Amylase.

Part XIII.—Effect of the Variety of Aspergillus Oryzae employed in Saké Manufacture upon the Formation of Amylases, Maltase and Protease in Saké-koji.

(pp. 414∼418)

By Yuzo Tokuoka.

(Tsunekichi Okura Brewery, Received Jan. 31, 1939.)

It was clearly pointed out that the cultural characteristics of Asp. oryzae and the external appearance of saké-koji (Asp. oryzae grown on steamed rice) were quite different according to the various straines of Asp. oryzae.

However, no remarkable change in the formation of enzyme including α- and β-amylases, maltase or protease in saké-koji was found, although the formation of α-amylase was suggested as being inversely proportional to the ratio of between the amounts of β- and α-amylases and of between maltase and α-amylase.

The relative activity of α- and β-amylases or maltase in various sakè-koji at 40° in pH 5.0 was found to be 100 : 19.5～11.2 (av. 15.4) : 9.3～6.2 (av. 7.5).

Therefore the relative activity of the above enzymes was again found to be 100 : $<$ 2 : $<$ 1 with such other koji-preparations as soy-bean, rice and wheat brans, since the relative activity of β-amylase and maltase was found to be about 10 times higher with saké-koji as mentioned in the previous paper [see This Journal. **14**, 839 (1938)].